REMARKS

I. Status of the Application

Claims 1-100 are pending in the application. Applicants gratefully acknowledge that the drawings filed on 24 July 2004 have been accepted by the Examiner. Claims 1-72 and 86-100 have been cancelled without prejudice to the filing of any appropriate continuation applications as being directed to non-elected subject matter. Claims 73-85 stand rejected under 35 U.S.C. § 112, first paragraph, as lacking enablement. Claims 73-85 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Mayford et al. (1996) *Science* 24:1678 and Ahlijanian et al. (2000) *Proc. Natl. Acad. Sci. USA* 97:2910, in view of Lucas et al. (2001) *The EMBO Journal* 20:27.

Applicants have amended the claims under consideration to more clearly define and distinctly characterize Applicants' novel invention. Specifically, Applicants have amended claims 73 and 85 to recite a calcium-calmodulin-dependent kinase II promoter, support for which can be found at least at claim 75 as originally filed.

The amendments presented herein contain no new matter. Applicants respectfully request entry and consideration of the foregoing amendments, which are intended to place the case in condition for allowance.

II. Claims 73-85 Are Enabled

At section 2 of the instant Office Action, claims 73-85 stand rejected under 35 U.S.C. § 112, first paragraph, because the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with the claims. Applicants respectfully traverse this rejection.

35 U.S.C. §112, first paragraph requires that the specification must enable a person skilled in the art to make and use the claimed invention. However, a specification need not, and should not, disclose what is well known in the art. The invention that one skilled in the art must be enabled to make and use is that defined by the claims of the particular application. The issue of adequate enablement depends on whether one skilled in the art could practice the claimed invention without undue experimentation. Enablement is not precluded by the necessity of some experimentation such as routine screening, ***even if it is extensive routine screening***. Also, the fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation (MPEP 2164.01) if the level of skill in the art is high or if all of the methods needed to practice the claimed invention are well known. *In re Wands*, 8 U.S.P.Q. 2d 1400, 1406 (Fed. Cir. 1988).

> The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art. (Citations omitted). The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. *In re Wands*, 8 U.S.P.Q. 2d at 1404.

The Office Action states that, given the lack of guidance provided by the instant specification, it would have required undue experimentation to practice the invention for the creation of a transgenic mouse overexpressing in the brain or forebrain a nucleic acid encoding p25 under an inducible Tetracycline (Tet) on/Tet off system wherein said mouse exhibits the pathological features associated with progressive neurodegeneration and the transgenic mouse exhibits behavioral symptoms of Alzheimer's disease without a reasonable expectation of success for the breadth of the claims (page 10).

Applicants disagree. Applicants' specification more than adequately teaches one of skill in the art to make and use the claimed mouse whose genome comprises a first transgene

comprising a DNA sequence encoding p25 operably linked to an inducible promoter, and a second transgene comprising a DNA sequence encoding an inducer operably linked to a calcium-calmodulin-dependent kinase II promoter.

Operably linking an inducible promoter to a DNA sequence encoding p25 would be routine to one of skill in the art based on the guidance of Applicants' disclosure and the knowledge in the art at the time of filing. Applicants teach that a p25 plasmid vector was known in the art at the time of filing (Patrick et al. (1999) *Nature* 402:615) (specification, page 64, first full paragraph). Applicants teach the use of inducible promoters (Specification, page 4, second full paragraph), and submit that inducible promoters suitable for use in the claimed transgenic mouse were well known in the art at the time of filing. For example, heat shock protein-, GAL4- and mifeprisone-inducible systems were known in the art (see abstracts set forth as Exhibits A-C, respectively). Further, promoters and their use were described in texts such as Goeddel, "Gene Expression Technology," *Methods in Enzymology* 185, Academic Press, San Diego, CA (1990) at the time of filing.

Applicants teach that the claimed mouse can be created by introducing a p25-encoding nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection or retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal, and that methods for generating Tg mice were conventional in the art at the time of filing and are described in publications such as U.S. Patent Nos. 4,736,866, 4,870,009 and 4,873,191, and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986) (specification, page 33, first paragraph). Applicants specifically teach how linearized a p25 plasmid vector and how to microinject the linearized p25 plasmid vector into fertilized mouse eggs (specification, page 33, first paragraph). Applicants teach that

a transgenic founder animal can be identified based upon the presence of a detectable translation product transgene in its genome and/or expression of detectable translation product mRNA in tissues or cells of the animals. *Id.* Applicants specifically teach how to screen founders by Southern blot and PCR analysis. *Id.* Applicants teach how to cross founders with CaMKII-tTA Tg mice that were known in the art (Mayford et al. (1996) *Science* 274:1678), and how to backcross mice to obtain a homogeneous C57BL/6J background. *Id.* Applicants teach that mice for analysis were derived from heterozygote crosses to ensure all genotypes in each litter, which were obtained in a Mendelian manner. *Id.* Applicants further teach that all mice were conceived (throughout gestation) and raised in the presence of doxycycline (up to one mg/g in food, changed twice a week) (Bio-Serv, Frenchtown, NJ) for at least 4-6 weeks postnatal before induction of p25 (by removal of doxycycline). *Id.*

Thus, based on Applicants' teachings and the knowledge in the art at the time of filing, one of skill in the art could easily make and use the claimed transgenic mouse with a reasonable expectation of success.

The Office Action states that the specification, while being enabling for a transgenic mouse whose genome comprises a nucleic acid sequence encoding the human p25 operably linked to an inducible tetracycline (Tet) Tet on/Tet off CaMKII promoter, and a second transgene comprising a DNA sequence encoding an inducer operably linked to the tissue-specific promoter, wherein p25 is expressed and wherein the mouse has neurodegenerative pathology, does not reasonably provide enablement for all tissue specific promoter Tet on/Tet off system transgenic mice ***that have behavioral symptoms of Alzheimer's disease*** (page 3, section 2 of instant Office Action).

Applicants disagree with this assertion. Applicants' claimed invention is directed to a

transgenic mouse whose genome comprises a first transgene comprising a DNA sequence encoding p25 operably linked to an inducible promoter, and a second transgene comprising a DNA sequence encoding an inducer operably linked to a calcium-calmodulin-dependent kinase II promoter. Thus, the claimed mouse expresses two transgenes, one, a DNA sequence encoding p25 operably linked to an inducible promoter, and the other, a DNA sequence encoding an inducer operably linked to a calcium-calmodulin-dependent kinase II promoter. Although the claimed mouse *may* have one or more features as set forth in dependent claim 82 (i.e., progressive neurodegeneration, tau aggregation, neurofibrillary tangle formation, aberrant cyclin-dependent kinase 5 activity, neuronal loss in the cerebral cortex, neuronal loss in the hippocampus, severe brain atrophy, reactive astrogliosis, caspase-3 activation, up-regulation of C99, up-regulation of beta-amyloid, tau hyperphosphorylation, amyloid precursor protein phosphorylation, and amyloid precursor protein hyperphosphorylation), it is not necessary that the mouse exhibit the ***behavioral*** symptoms of Alzheimer's disease. All that is required is that the claimed elements are present.

The Office Action states, at page 9, that the robust tau phosphorylation phenotypes seen and claimed for in the present mouse were not seen using the NSE promoter used by Ahlijanian et al. The Office Action then concludes that the ***phenotypes*** of the presently claimed mouse are ***dependent*** on the ***Tet on/Tet off inducible system.*** Applicants respectfully disagree. Ahlijanian et al. does indeed observe tau phosphorylation: "The overexpression of an activator of cdk5 in transgenic mice results in increased cdk5 activity that is ***sufficient to produce hyperphosphorylation of tau***" (abstract, emphasis added). Indeed, Ahlijanian et al. histologically demonstrates hyperphosphorylation of tau in the amygdala, thalamus /hypothalamus and cerebral cortex in their transgenic mice using the phospho-specific

monoclonal antibodies AT-8 and PHF-13 (see paragraph bridging pages 2911 and 2912 and Figure 2). The wild-type mice demonstrated no such staining. Although Ahlijanian et al. do not detect altered tau phosphorylation by immunoblotting, they state that this was likely due to the fact that the fraction of neurons positive for silver staining in the transgenic mice was relatively low, approaching 5% (page 2914, first full paragraph). An immunoblot assay would likely not be sensitive enough to detect such a low percentage of phosphorylated tau. Ahlijanian et al. concludes that, based on the positive immunostaining, the local concentrations of the phospho-tau epitopes in immunopositive neurons are likely to be relatively high (page 2914, first full paragraph). Further, the transgenic mice of Ahlijanian et al. demonstrated physical disturbances and behavioral alterations similar to those observed in neurodegenerative diseases such as Alzheimer's disease (page 2915, first full paragraph). Thus, the Office Action's conclusion that the phenotypes of the presently claimed mouse are dependent on the Tet on/Tet off inducible system is simply not supported by the teachings of Ahlijanian et al.

For at least the reasons set forth above, Applicants' specification, coupled with the level of skill in the art, enables a person of skill in the art to make and/or use the claimed transgenic mouse. Accordingly, the Examiner is respectfully requested to reconsider and withdraw the rejection of claims 73-85 under 35 U.S.C. § 112, first paragraph, as lacking enablement.

III. Claims 73-85 Are Nonobvious over Mayford et al. and Ahlijanian et al. in view of Lucas et al.

At section 3 of the instant Office Action, claims 73-85 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Mayford et al. (1996) *Science* 24:1678 and Ahlijanian et al. (2000) *Proc. Natl. Acad. Sci USA* 97:2910, in view of Lucas et al. (2001) *The EMBO Journal* 20:27. The Office Action states that, in view of the teachings of Ahlijanian et al. taken with the

teachings of Lucas et al., it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the Tet on/Tet off system of Mayford et al., by using the p25 transgenic mouse technology to monitor spatial and temporal elevations of the p25 levels under tightly controlled inducible expression systems and study the associated neurodegenerative pathology and behavioral phenotypes with a reasonable expectation of success. The Office Action further states that one of ordinary skill in the art would have been motivated to make such a modification as it was recognized by Ahlijanian et al. and Lucas et al. the need to determine the spatial and temporal relationship of neurodegenerative pathology to the behavioral phenotypes of AD. Applicants respectfully traverse this rejection based on the amended claims now presented.

The pending claims are directed to a transgenic mouse whose genome comprises a first transgene comprising a DNA sequence encoding p25 operably linked to an inducible promoter, and a second transgene comprising a DNA sequence encoding an inducer operably linked to a calcium-calmodulin-dependent kinase II promoter. Applicants' claimed mouse is useful for studying the effects of p25 overexpression *in vivo*. Specifically, the claimed mouse provides exquisite control of p25 overexpression in the brain and, accordingly, is useful for studying a variety of disorders caused or exacerbated by p25 overexpression in the brain. Such disorders can include neurodegenerative disorders such as Alzheimer's disease.

Applicants respectfully submit that motivation does not exist to modify Mayford et al. in the manner suggested by the Office Action to arrive at Applicants' claimed subject matter with a reasonable expectation of success. Applicants respectfully submit that the mere fact that references can be combined does not render the resultant combination obvious unless the prior art also ***suggests the desirability of the combination***.

Mayford et al. teaches a transgenic mouse overexpressing a CaMKII transgene under the control of the Tet on/Tet off system. Mayford et al. is looking at the effects of gene expression on memory. Mayford et al. states that "genetic modifications often affect, indiscriminately, both implicit and explicit memory as well as perceptual or motor performance" (page 1678, left column, second paragraph. Importantly, Mayford et al. teaches, "to analyze the molecular contribution of a given gene to a particular type of memory, ***it is essential*** not only ***to control the timing of expression*** but also to restrict expression to appropriate cell populations" (page 1678, left column, second paragraph, emphasis added). Thus, Mayford et al. uses an inducible promoter because it is ***necessary*** in order to analyze the pathways of memory. Mayford et al. neither teaches nor suggests controlling p25 expression using an inducible promoter.

Ahlijanian et al. teaches p25 transgenic mice overexpressing p25. The transgenic p25 mice of Ahlijanian et al. show tau and neurofilament hyperphosphorylation, display silver-positive neurons using the Bielschowsky stain, demonstrate disturbances in neuronal cytoskeletal organization in the amygdala, thalamus/hypothalamus and cortex, display increased spontaneous locomotor activity and differences from control in the elevated plus-maze test (abstract). Ahlijanian et al. concludes that their transgenic mice produce hyperphosphorylation of tau and neurofilament as well as cytoskeletal disruptions reminiscent of Alzheimer's disease and other neurodegenerative diseases (abstract). Ahlijanian et al. does not teach or suggest the use of an inducible promoter, nor does it teach or suggest use of the claimed calcium-calmodulin-dependent kinase II promoter. Given their success in generating neurodegenerative mice without the use of an inducible promoter and their lack of a teaching or suggestion to use one, one of skill in the art, based on the teachings of Ahlijanian et al., would find no motivation to generate a construct for expressing p25 under the control of an inducible promoter.

The Office Action reasons that Lucas et al. provides sufficient motivation for one of skill in the art to apply the Mayford et al. inducible Tet on/Tet off system to create a transgenic mouse overexpressing p25 taught by Ahlijanian et al. Applicants respectfully submit that Lucas et al. provides no such motivation. Lucas et al. teaches the use of an inducible Tet system in order to express a gene that was known to ***cause perinatal lethality*** due to ***toxicity of the transgene*** (page 28, left column, third full paragraph). In contrast, p25 is not lethal during embryonic development. Indeed, Ahlijanian et al. reported ***no lethality*** when p25 was constitutively overexpressed in mice. Because p25 is not lethal during development, one of skill in the art, based on the teachings of Lucas et al., would find absolutely no motivation to modify the primary references in order to arrive at the claimed invention.

For at least these reasons, the combination of Mayford et al. and Ahlijanian et al. in view of Lucas et al. fails to render the claimed invention obvious. Accordingly, Applicants respectfully request that the rejection of claims 73-85 under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

IV. CONCLUSION

Having addressed all outstanding issues, Applicants respectfully request reconsideration and allowance of the case. To the extent the Examiner believes that it would facilitate allowance of the case, the Examiner is requested to telephone the undersigned at the number below.

Respectfully submitted,

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1: Cancer Res. 2000 Mar 15;60(6):1637-44.

Photodynamic therapy-mediated oxidative stress as a molecular switch for the temporal expression of genes ligated to the human heat shock promoter.

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Oxidative stress associated with photodynamic therapy (PDT) is a transcriptional inducer of genes encoding stress proteins, including those belonging to the heat shock protein (hsp) family. The efficiency of PDT to function as a molecular switch by initiating expression of heterologous genes ligated to the human hsp promoter was examined in the present study. Selective and temporal reporter gene expression was documented after PDT in mouse radiation-induced fibrosarcoma cells stably transfected with recombinant vectors containing an hsp promoter ligated to either the lac-z or CAT reporter genes and in transfected radiation-induced fibrosarcoma tumors grown in C3H mice. Hyperthermia treatments were included as a positive control for all experiments. Expression vectors containing either human p53 or tumor necrosis factor (TNF)-alpha cDNA under the control of an hsp promoter were also constructed and evaluated. A p53 null and TNF-alpha-resistant human ovarian carcinoma (SKOV-3) cell line was stably transfected with either the p53 or TNF-alpha constructs. Inducible expression and function of p53 as well as inducible expression, secretion, and biological activity of TNF-alpha were documented after PDT or hyperthermia in transfected SKOV cells. These results demonstrate that PDT-mediated oxidative stress can function as a molecular switch for the selective and temporal expression of heterologous genes in tumor cells containing expression vectors under the control of an hsp promoter.

PMID: 10749134 [PubMed - indexed for MEDLINE]

1: Mol Ther. 2001 Mar;3(3):278-83.

Augmenting transgene expression from carcinoembryonic antigen (CEA) promoter via a GAL4 gene regulatory system.

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Though extensively studied, the use of tissue- or cell-type-specific promoters to target transgene expression is hampered by their weak activity. We hypothesized that this problem could be addressed by using a GAL4 gene regulatory system, wherein a weak, tissue-specific promoter would drive expression of the GAL4/VP16 fusion protein (GV16), which in turn would transactivate a minimal synthetic promoter, GAL4/TATA (GT), upstream of a transgene. To test this hypothesis, we constructed adenoviral vectors expressing a lacZ or GV16 gene driven by a carcinoembryonic antigen (CEA) promoter (Ad/CEA-LacZ or Ad/CEA-GV16) and evaluated levels of transgene expression they produced in cultured cells and in subcutaneous tumors after intratumoral administration. In CEA-positive cells, treatment with Ad/CEA-GV16 + Ad/GT-LacZ versus Ad/CEA-LacZ increased transgene expression 20- to 100-fold. In CEA-negative cells, treatment with Ad/CEA-GV16 + Ad/GT-LacZ increased transgene expression to a much lower degree (6- to 8-fold). In addition, analysis of Bax gene-mediated cell death revealed that this system can be used to avoid Bax's toxic effects on CEA-negative cells without compromising its ability to kill CEA-positive cells in vitro and in vivo. Thus, the combination of a tissue-specific promoter with the GAL4 gene regulatory system could be useful for targeting transgene expression.

PMID: 11273768 [PubMed - indexed for MEDLINE]

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1: Mol Endocrinol. 2000 Jul;14(7):1075-85.

Regulable expression of inhibin A in wild-type and inhibin alpha null mice.

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Exogenous regulation of protein expression creates the potential to examine the consequences of homeostatic Dysregulation in many physiological systems and, when used in transgenic mice, provides the capability of restoring a gene product to its knockout background without antigenicity issues. In this study, we used a mifeprisone-inducible system (the GeneSwitch system) to regulate the expression of inhibin A from the liver of mice. Inhibin is a heterodimeric protein (alpha/beta) wherein one of its subunits (beta) is capable of homodimerizing to form its physiological antagonist, activin (beta/beta). Inhibin is also expressed in two forms, A and B, as determined by the subtype of beta-subunit that dimerizes with the alpha-subunit (alpha/betaA or alpha/betaB). To utilize the GeneSwitch system, transgenic transactivator mice with liver-specific expression of a mifepristone-activated chimeric nuclear receptor (GLVP) were crossed with transgenic target mice containing a GVLP-responsive promoter upstream of polio-virus IRES (internal ribosome entry site)-linked sequences coding for the alpha- and beta-subunits of inhibin A. This intercross produced "bigenic" mice capable of regulable expression of inhibin A from the liver. Overexpression of inhibin A in wild-type mice produced a phenotype wherein males had decreased testis size and females had a block in folliculogenesis at the early antral stage, findings similar to activin type IIA receptor (ActRIIA) null mice. These phenotypes were most likely due to suppressed serum FSH, confirming that the liver-derived inhibin A was secreted into the serum to down-regulate pituitary FSH levels. Furthermore, the generation of bigenic mice in the inhibin alpha null background allowed for the induction of inhibin A in inhibin alpha null male mice with subsequent rescue of these mice from their gonadal tumor-induced lethal phenotype. This work demonstrates the in vivo production of a heterodimeric hormone from a single inducible promoter to study its therapeutic and physiological effects. In addition, these studies are the first example of an inducible system being used to prevent a lethal knockout phenotype in an animal model.

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